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RESEARCH ARTICLE

EVALUATION OF ANTIDIABETIC AND ANTIINFLAMMATORY ACTIVITIES OF A POLYHERBAL FORMULATION.

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ABSTRACT

: The present study was taken with an objective of Evaluation of antidiabetic and Antiinflammatory activities of Polyherbal Formulation GLUCOSTAT. In this study the antidaibetic activity of PHF Glucostat was evaluated by using Alloxan Induced diabetes in rats model and Anti inflammatory activity was evaluated by Carragenan induced paw edema in rats model. In the Evaluation of antidiabetic activity the biochemical parameters of Glucose, Cholesterol, Triglycerides, LDL and HDL were estimated in the diabetes induced rats and compared among control and treatment groups proving that the PHF Glucostat had the potential antidiabetic activity. In the model of Carragenan induced paw edema model in rats the paw volume was measured at different intervels and compared among the control and treatment groups concludes that the PHF Glucostat possessing antiinflammatory activity

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism. There are estimated 143 million people worldwide sufferings from diabetes, almost five times more than the estimates ten years ago. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in south-east Asia and western Pacific being most at risk[1]. It has been estimated that in 1995, 19.4 million individuals were affected by diabetes mellitus in India and these numbers are expected to increase to 57.2 million by the year 2025 (one sixth of the world)[2]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown[3].

Inflammation is the body's immediate response to damage to its tissues and cells by pathogens, noxious stimuli such as chemicals, or physical injury[4]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation can be classified as either acute or chronic status depending on onset time. Although inflammation is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders like arthritis, cancer, asthma and diabetes. Usually the diabetes occurs in the people at age of around 40 years and these people always complain pains and inflammations also. There are many allopathic medicines are available to manage these two symptoms separately.

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Glucostat which is a Ploy herbal formulation (PHF) used as Antidiabetic & Anti – inflammatory agent. Based on the ancient history of the ingredients of Glucostat, it was used to treat madhumeha (Diabetes) and anti inflammatory agent.

So an attempt has been made with an objective to evaluate the effectiveness of the PHF Glucostat in diabetic condition in rats and anti inflammatory activities in rats.

MATERIAL AND METHODS

Drugs and chemicals:

The chemicals & reagents used for this study were analytical grade, Metformin was a gift sample from Micro labs and all the kits used to evaluate the blood parameters were procured from Span Diagnostics Ltd.

Dose selection and preparation[5]

Poly Herbal Formulation GLUCOSTAT available in the form Capsules. Each 500 mg capsule contains 450 mg ingredients. The Human Dose for the Glucostat is 06 capsules per a day. i.e 06 X 450 mg = 2,700 mg/day. The conversion factor for conversion of Human (70 kg.) dose to Rat (200 gm) dose is 0.018.

2700 mg (Human Dose 70 kg.) X 0.018 = 48.6 mg/200 gm Rat i.e 243 mg/ Kg body weight of Rat. We will make this 243 mg to round figure 250 mg/ kg body weight. This 250 mg/Kg body weight was considered as Lower dose and double of this dose 500 mg/kg bodyweight was considered as higher dose.

ANTIDIABETIC ACTIVITY:

ALLOXAN INDUCED DIABETES IN RATS[6].

Male Wistar albino rats weighing between 170- 190 gms were brought from NIN Hyderabad; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

Induction of Diabetes in animals:

Rats were made diabetic by a single intra peritoneal injection of Alloxan monohydrate dissolved in normal saline with a dose of 150 mg/kg. Two days after Alloxan injection, rats with plasma glucose levels more than 140 mg/dl were included in the study and devided into 04 groups of 06 animals in each group. Treatment was started with drugs 48 hrs after the Alloxan injection. The treatment and grouping were done as follows. The treatment will be continued for 14 days.

Group 01: Normal control : The animals were not treated with Alloxan and received vehicle (2% w/v acacia Suspension) at dose 10ml/kg body weight.

Group 02: Diabetic Control : The animals were treated with Alloxan 150 mg/kg and vehicle (2% w/v acacia suspension) at dose 10 ml/kg body weight.

Group 03: The animals were treated with Alloxan 150 mg/kg and received PHF Glucostat lower dose (250 mg/kg) in the vehicle orally.

Group 04: The animals were treated with Alloxan 150 mg/kg and received PHF Glucostat Higher dose (500 mg/kg) in the vehicle orally.

Group 05: standard group : The animals were treated with Alloxan 150 mg/kg and received Glibenclamide (5 mg/kg) in the vehicle orally (reference standard)

Blood samples were collected by puncturing retro orbital plexus on 01, 07 and 14 day of study and analyzed for serum glucose and on day 14 along with serum glucose, serum triglycerides, serum cholesterol, HDL & LDL were estimated.

ANTI INFLAMMATORY ACTIVITY :

CARRAGEENAN INDUCED HIND PAW EDEMA IN RATS[7]

Albino Wistar rats weighing between 150-200gms were divided into 4 groups of 6 rats each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum. The grouping of animals is as follows.

Group I: Animals were administered 0.1ml saline p.o

Group II:Animals were administered 0.1ml saline p.o

Group III:Animals were administered Glucostat (250 mg/kg) p.o

Group IV:Animals were administered Glucostat (500 mg/kg) p.o

Group III:Animals were administered standard (Indomethacin 10 mg/kg) p.o

Procedure:

All rats of II, III IV & V (except I group) groups were injected with 0.1ml of carageenan (1%) in normal saline into sub planter area of right hind paw. All the drugs were given orally 1hr prior to Carrageenan injection. Paw volume was measured by mercury plethysmograph at 0, 30, 60, 90, 120 minutes after the Carrageenan injection.

STATISTICAL ANALYSIS:

All the data's were analyzed using One-Way ANOVA method followed by Dunnet's / Tukey's test. All values were reported as mean ± SEM. P≤0.05 was considered to be statistically significant.

RESULTS

Antidiabetic activity

Alloxan induced diabetes in rats:

The PHF Glucostat showed significant anti diabetic activity on 07th & 14th day at doses of 250 mg/kg and 500 mg/kg. The blood glucose levels in the animals treated with doses of 250 mg/kg and 500 mg/kg groups were found to be 169.65±5.8mg/dl & 148.42±6.3 mg/dl respectively on 07th day and 162.52±7.4 & 142.78±6.7 respectively on 14th day and found significant when compared with diabetic control group . The blood glucose levels in the animals treated with Glibenclamide (5 mg/kg) group were found to be 139.64±4.8mg/dl & 140.28±10.5 on 07th & 14th days respectively and found significant when compared with diabetic control group

Table .1: Effect of GLUCOSTAT *on* blood glucose (mg/dl) levels in Alloxan induced

Diabetes in Rats model

Treatment	**Blood glucose levels (mg/dl)**		
	01st day	**07th day**	**14th day**
Control	135.51 ±8.2	136.68±8.2	137.89±3.8
Diabetic control	262.11 ±6.2^{+++}	271.64 ±4.4^{+++}	251.86±4.8^{+++}
Glucostat (250 mg/kg)	245.51±3.9^{ns}	169.65±5.8**	162.52±7.4***
Glucostat (500 mg/kg)	234.67±4.8 ^{ns}	148.42±6.3***	142.78±6.7***
Glibenclamide (5 mg/kg)	216.26±6.8*	139.64±4.8***	140.28±10.5***

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Tukey's test.
^{ns} P>0.005 Vs Diabetic control, * P< 0.005 Vs Diabetic control ** P< 0.01 Vs Diabetic control, *** P< 0.001 Vs Diabetic control.
$^{+++}$P<0.0001 Vs Control.

Table .2: Effect of PHF GLUCOSTAT on Serum Triglycerides, Cholesterol, HDL & LDL levels in Alloxan induced Diabetes in Rats model

Treatment	**Triglycerides (mg/dl)**	**Cholesterol (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**
Control	64.8 ± 1.675	62.7 ± 1.065	38.7 ± 0.682	91.6 ± 1.245
Diabetic control	182.3± 3.564^{+++}	132.7 ± 1.524^{+++}	24.8 ± 0.574^{+++}	176.7 ± 1.342^{+++}
Glucostat (250 mg/kg)	105.7±1.874**	117.6 ± 1.257**	29.4 ± 0.496**	148.5 ± 1.268**
Glucostat (500 mg/kg)	98.6 ± 1.487***	98.6 ± 1.548***	34.6 ± 0.621***	111.4 ± 1.387***
Glibenclamide (5 mg/kg)	89.5 ± 1.247***	79.5 ± 1.267***	36.7 ± 0.512***	97.8 ± 1.287***

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Tukey's test.
** P< 0.01 Vs Diabetic control, *** P< 0.001 Vs Diabetic control.
+++P<0.0001 Vs Control.

From the above table it was observed that, the biochemical parameters such as Triglycerides, Cholesterol and LDL levels were elevated along with the glucose levels with the induction of diabetes, which was observed in the Diabetic control group. But after the completion of treatment with Glucostat and Glibenclamide to III, IV and V groups the elevated levels of Triglycerides, Cholesterol and L were significantly brought towards normal values. In case of HDL levels, they were decreased with induction of Diabetes but after the effective treatment with Clove extract and Glibenclamide the reduced HDL levels were elevated significantly and brought towards the normal levels.

Anti – inflammatory activity:

Carrageenan induced paw edema in rats:

In Carrageenan induced paw edema PHF GLUCOSTAT significantly inhibited the edema in a dose dependent manner as shown in Table.4. The paw volume in inflammatory control group rats at 60 mins. was found to be 0.2148 ± 0.0122 ml. The paw volume in rats pretreated with Clove extract (100 mg/kg & 200 mg/kg), and Indomethacin (10 mg/kg/day) at 60 mins. were found to be 0.1568 ± 0.0051 ml, 0.1486 ± 0.0057 and 0.1369 ± 0.0054 ml respectively.

Table 3: Anti-inflammatory effect of PHF GLUCOSTAT on Carrageenan induced paw edema in rats

Treatment	Paw volume in ml at different Hrs (Mean ± S.E.M.)				
	0 min	30 min	60 min	90 min	120 min
Normal Control	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058
Inflammatory controlControl	0.1225 ± 0.0079+++	0.1876 ± 0.007+++	0.2148 ± 0.0122+++	0.2083 ± 0.0094+++	0.165 ± 0.0076+++
Indomethacin 10mg/kg, p.o.	0.1249 ± 0.0061	0.1389 ± 0.0049**	0.1369 ± 0.0054***	0.1442 ± 0.007***	0.1449 ± 0.0060***
Glucostat (250 mg/kg)	0.1268 ± 0.0067	0.1427 ± 0.0071**	0.1568 ± 0.0051***	0.1554 ± 0.0068***	0.1549 ± 0.0098**
Glucostat (500 mg/kg)	0.1116 ± 0.0085	0.1489 ± 0.0068**	0.1486 ± 0.0057***	0.1589 ± 0.0076***	0.1572 ± 0.0087***

Values are expressed as (Mean±SEM) n=6; One way ANOVA followed by Tukey's test. +++ P<0.001 Vs Normal control ** P< 0.01 Vs Inflammatory Control & *** P< 0.001 Vs Inflammatory Control

DISCUSSION:

In the light of results, our study indicates that PHF GLUCOSTAT have good Antidiabetic activity. PHF GLUCOSTAT exhibited significant anti hyperglycemic activity in alloxan induced diabetic rats. The rise in the blood glucose level is accompanied with the increase in TG, TC, LDL, and fall in HDL. This is due to the insulin deficiency, which results in the faulty glucose utilization and mobilization of fatty acids from adipose tissue[8]. The purpose of fatty acid breakdown is to meet the energy requirements; this would lead to high free fatty acids in plasma. The marked hyperglycaemia that characterizes the diabetic state may therefore be regarded as consequences of the uninhibited actions of lipolytic hormones on the fat depots.Chronic insulin deficiency might be associated with a diminished level of LDL receptors this causes the increase in LDL particles and in results in LDL cholesterol in diabetes mellitus. The probable mechanism of action of PHF GLUCOSTAT could be the increased the sensitivity of the insulin towards it's receptors and also may be the increased insulin levels and increased mass of beta cells of pancreas because the previous studies indicates that, the alloxan will induce the diabetes by damaging the beta cells[9]. This study

needs further research to establish the mechanism of action of PHF GLUCOSTAT at molecular level.

The development of edema in the paw of the rat after injection of Carrageenan is a biphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances[10]. Inhibition of edema observed in various inflammatory models induced experimentally in the present study may, therefore be attributed to the ability of the PHF GLUCOSTAT to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase.

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